Common Volatiles Analysis by Headspace GC-MSD/FID

1 Introduction

This procedure allows for the screening, identification, confirmation, and quantitation of common volatile chemicals.

2 Scope

Analyses	☒ Screening ☒ Confirmation ☒ Quantitation
Matrices	Blood, serum, urine, vitreous fluid and other liquids (0.1 mL per analysis)
Analytes	Ethanol, acetone, isopropanol, methanol (Target Compounds)
Personnel	This document applies to Chemistry Unit case working personnel who perform toxicology analyses.

3 Principle

Sample and a diluent containing internal standard are added to a headspace vial using a pipette operating in dilute mode. Samples are qualitatively screened for target compounds by headspace gas chromatography with mass spectrometry (HS-GC/MS). Specimens are quantitatively confirmed through a separate analysis using headspace gas chromatography with flame ionization detection (HS-GC/FID). The headspace technique is based on Henry's gas law which states that when a volatile chemical in solution, such as ethanol, comes into contact with a closed air space, an equilibrium forms between the liquid phase and the headspace. At a constant temperature, the partial pressure of the volatile chemical in the headspace is directly proportional to its concentration in solution. This method provides a means of analyte separation from the matrix and produces a chemical in the vapor state ready for gas chromatographic analysis.

4 Procedure

4.1 Screening/Identification by HS-GC/MSD

Batch Building: Samples used for screening may be aliquoted in advance up to 15 days prior to analysis (samples are portioned into a headspace vial, sealed, and placed in secure refrigerated storage). The same lot of Sample Diluent is used throughout a given batch. Any calibrated Xplorer Plus pipette may be used. Forensic Advantage (FA) is used to track the samples in a given batch. Batches are coded according to the scheme TOX200.YYYYMMDD. Sample portions are tracked in FA as subdivided evidence (e.g., "Item 1-1"). Headspace vials may be identified with a barcode label or other means.

Step		Activity	Note	Reference/Lot
4.1.1	☐	**Samples:** Using an Eppendorf Xplorer pipette fitted with a tip, aliquot 0.8mL of Sample Diluent and 0.1mL of sample into a 10 mL headspace vial. Crimp vial firmly using a magnetic cap. Use a new tip for each sampling.	See pipette settings in Section 5.1	S^3 Sample Diluent Xplorer Plus Pipette 3
4.1.2	☐	**Quality Control Materials:** To start a batch, pipet the following QC materials: • Negative Control (deionized water) • 0.010 g% (CRM) • 0.200 g% (CRM) Upon aliquot of the final case sample for the batch, include a closing control: • 0.100 g% (CRM)		Negative Control Calibrator C1-C6 Set 2
4.1.3	☐	**Batch Analysis:** Scan (or input) the samples into the instrument sequence using the suggested following order and format: A maximum of 116 samples may be analyzed in one batch.		

	Vial	Sample Type	Sample Name	Method File	Data Path	Data File	Tray	Volume
1	1	Sample	NEG	TOX200-MSD.M	D:\MassHunter\Data\TOX200\20201019	20201019MSD-01	Rack 1,R60/10-CVM	1000.0
2	2	Cal	CAL 0.010	TOX200-MSD.M	D:\MassHunter\Data\TOX200\20201019	20201019MSD-02	Rack 1,R60/10-CVM	1000.0
3	3	Cal	CAL 0.200	TOX200-MSD.M	D:\MassHunter\Data\TOX200\20201019	20201019MSD-03	Rack 1,R60/10-CVM	1000.0
4	4	Sample	Case Sample 1	TOX200-MSD.M	D:\MassHunter\Data\TOX200\20201019	20201019MSD-04	Rack 1,R60/10-CVM	1000.0
5	5	Sample	Case Sample 2	TOX200-MSD.M	D:\MassHunter\Data\TOX200\20201019	20201019MSD-05	Rack 1,R60/10-CVM	1000.0
6	6	Sample	Case Sample 3	TOX200-MSD.M	D:\MassHunter\Data\TOX200\20201019	20201019MSD-06	Rack 1,R60/10-CVM	1000.0
7	7	Sample	Case Sample 4	TOX200-MSD.M	D:\MassHunter\Data\TOX200\20201019	20201019MSD-07	Rack 1,R60/10-CVM	1000.0
8	8	Sample	Case Sample 5...	TOX200-MSD.M	D:\MassHunter\Data\TOX200\20201019	20201019MSD-08	Rack 1,R60/10-CVM	1000.0
9	9	QC	CONTROL 0.10	TOX200-MSD.M	D:\MassHunter\Data\TOX200\20201019	20201019MSD-09	Rack 1,R60/10-CVM	1000.0

4.2 Confirmation/Quantitation by HS-GC/FID

Batch Building: Allow specimens and quality control samples to stand at room temperature for at least 15 minutes. Samples used for confirmation/quantitation are aliquoted from the original item into a headspace vial and sealed. The same lot of Sample Diluent is used throughout a given batch. Any calibrated Xplorer Plus pipette may be used. Forensic Advantage (FA) may be used to track the samples in a given batch. Batches are coded according to the scheme TOX200.YYYYMMDD. Sample portions may be tracked in FA as subdivided evidence (e.g., "Item 1-1). Headspace vials may be identified with a barcode label or other means.

Step		Activity	Note	Reference/Lot
4.2.1	☐	**Samples:** Using an Eppendorf Xplorer pipette fitted with a filter tip, aliquot 0.8mL of Sample Diluent and 0.1mL of sample into a 10 mL headspace vial. Crimp vial firmly using a magnetic cap. Perform in duplicate. Use a new tip for each sampling.	See pipette settings in Section 5.1	S^3 Sample Diluent Xplorer Plus Pipette 3
4.2.2	☐	**Quality Control Materials:** Use the following QC materials for each batch: • Negative Control (deionized water) • CAL1-CAL6, CAL7 (CRM) • Cliniqa Controls (Two Levels)		Negative Control Calibrator C1-C6 Set Calibrator C7 Cliniqa Level 1 Cliniqa Level 2 5
4.2.3	☐	**Batch Analysis:** Scan (or input) the samples into the instrument sequence using the suggested following order and format:		

	Vial	Sample Type	Sample Name	Method File	Data Path	Data File	Tray	Volume
1	1	Sample	Negative QC	TOX200-FID.M	D:\MassHunter\Data\TOX200\20201019	20201019FID-01	Rack 1,R60/10-CVM	1000.0
2	2	Cal	CAL1	TOX200-FID.M	D:\MassHunter\Data\TOX200\20201019	20201019FID-02	Rack 1,R60/10-CVM	1000.0
3	3	Cal	CAL2	TOX200-FID.M	D:\MassHunter\Data\TOX200\20201019	20201019FID-03	Rack 1,R60/10-CVM	1000.0
4	4	Cal	CAL3	TOX200-FID.M	D:\MassHunter\Data\TOX200\20201019	20201019FID-04	Rack 1,R60/10-CVM	1000.0
5	5	Cal	CAL4	TOX200-FID.M	D:\MassHunter\Data\TOX200\20201019	20201019FID-05	Rack 1,R60/10-CVM	1000.0
6	6	Cal	CAL5	TOX200-FID.M	D:\MassHunter\Data\TOX200\20201019	20201019FID-06	Rack 1,R60/10-CVM	1000.0
7	7	Cal	CAL6	TOX200-FID.M	D:\MassHunter\Data\TOX200\20201019	20201019FID-07	Rack 1,R60/10-CVM	1000.0
8	8	Cal	CAL7	TOX200-FID.M	D:\MassHunter\Data\TOX200\20201019	20201019FID-08	Rack 1,R60/10-CVM	1000.0
9	9	Sample	blank	TOX200-FID.M	D:\MassHunter\Data\TOX200\20201019	20201019FID-09	Rack 1,R60/10-CVM	1000.0
10	10	QC	Low QC	TOX200-FID.M	D:\MassHunter\Data\TOX200\20201019	20201019FID-10	Rack 1,R60/10-CVM	1000.0
11	11	QC	High QC	TOX200-FID.M	D:\MassHunter\Data\TOX200\20201019	20201019FID-11	Rack 1,R60/10-CVM	1000.0
12	12	Sample	blank	TOX200-FID.M	D:\MassHunter\Data\TOX200\20201019	20201019FID-12	Rack 1,R60/10-CVM	1000.0
13	13	Sample	Case 1	TOX200-FID.M	D:\MassHunter\Data\TOX200\20201019	20201019FID-13	Rack 1,R60/10-CVM	1000.0
14	14	Sample	Case 1	TOX200-FID.M	D:\MassHunter\Data\TOX200\20201019	20201019FID-14	Rack 1,R60/10-CVM	1000.0
15	15	Sample	blank	TOX200-FID.M	D:\MassHunter\Data\TOX200\20201019	20201019FID-15	Rack 1,R60/10-CVM	1000.0
16	16	Sample	Case 2	TOX200-FID.M	D:\MassHunter\Data\TOX200\20201019	20201019FID-16	Rack 1,R60/10-CVM	1000.0
17	17	Sample	Case 2...	TOX200-FID.M	D:\MassHunter\Data\TOX200\20201019	20201019FID-17	Rack 1,R60/10-CVM	1000.0
18	18	Sample	blank	TOX200-FID.M	D:\MassHunter\Data\TOX200\20201019	20201019FID-18	Rack 1,R60/10-CVM	1000.0
19	19	QC	Low QC	TOX200-FID.M	D:\MassHunter\Data\TOX200\20201019	20201019FID-19	Rack 1,R60/10-CVM	1000.0
20	20	QC	High QC	TOX200-FID.M	D:\MassHunter\Data\TOX200\20201019	20201019FID-20	Rack 1,R60/10-CVM	1000.0

A maximum of 35 items may be analyzed in one batch.

4.3 Screening or Confirmation for Nonstandard Samples

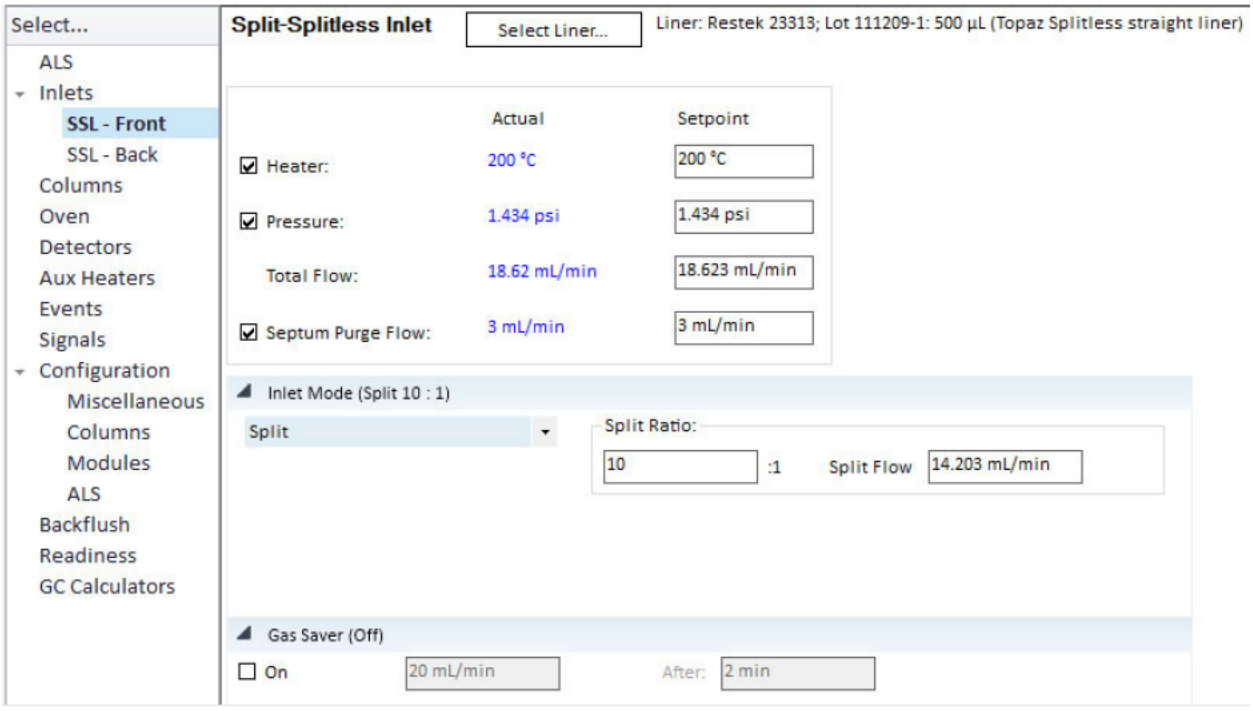
A sample is considered nonstandard if it cannot be rendered homogenous through mixing/vortexing, which are the preferred methods. If a case sample is clotted and cannot be pipetted accurately, it may be homogenized with a clot grinder before pipetting. If the values obtained from screening indicate that the sample analyte quantitated concentrations will exceed the method's calibration range, the analyst may dilute the sample in deionized water prior to sampling. However, this is not required.

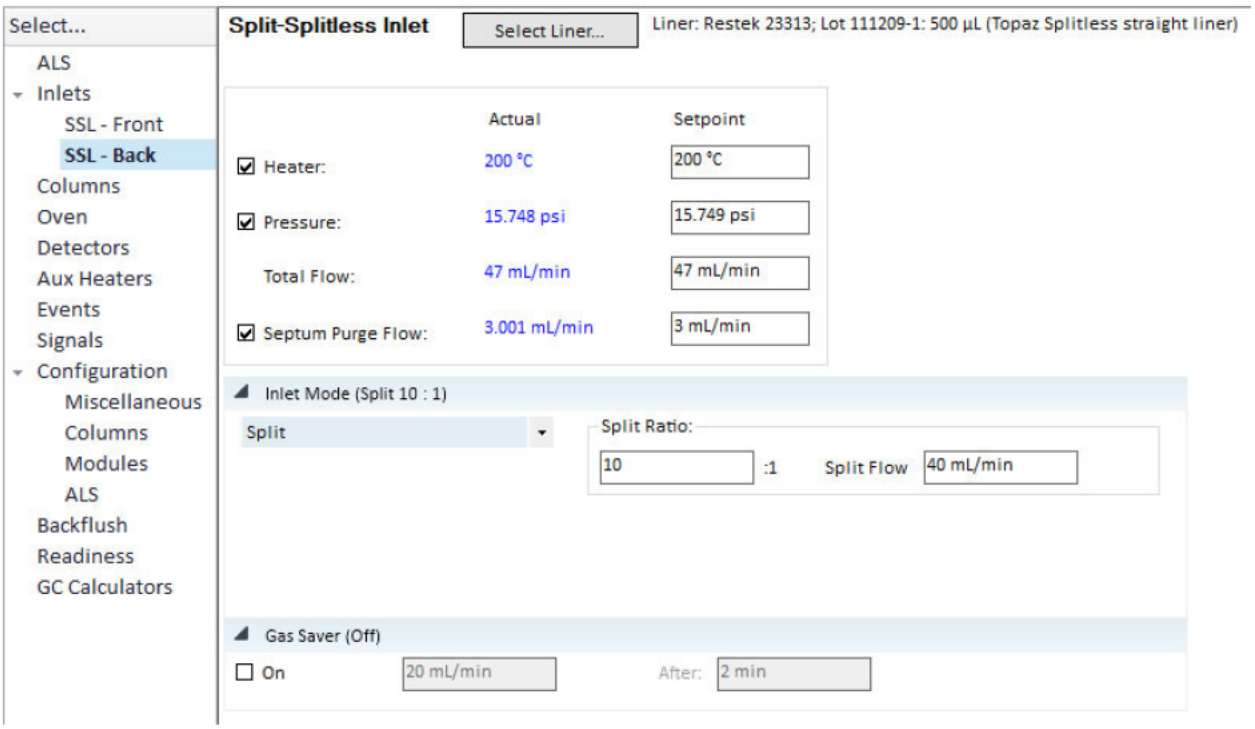
5 Instrument Parameters

5.1 Pipettor Settings (MSD and FID)

5.2 Mass Spectrometry Method (Screening)

5.2.1 Inlets

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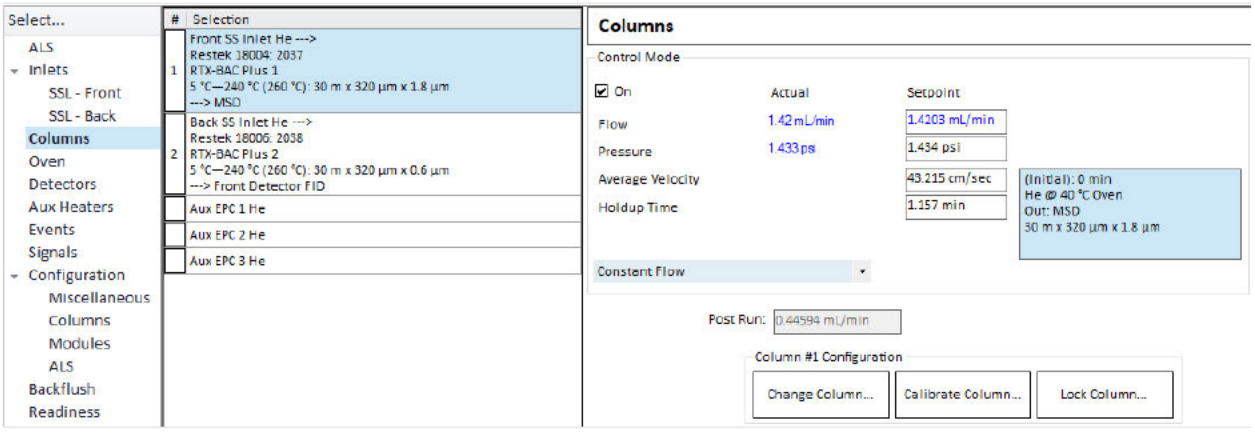
Inlets
SSL - Front
SSL - Back
Columns
Oven
Detectors
Aux Heaters
Events
Signals
Configuration
Miscellaneous
Columns
Modules
ALS
Backflush
Readiness
GC Calculators
Split-Splitless Inlet
Select Liner...
Liner: Restek 23313; Lot 111209-1: 500 µL (Topaz Splitless straight liner)
Actual
Setpoint
Heater:
200 °C
200 °C
Pressure:
15.748 psi
15.749 psi
Total Flow:
47 mL/min
47 mL/min
Septum Purge Flow:
3.001 mL/min
3 mL/min
Inlet Mode (Split 10 : 1)
Split
Split Ratio:
10
:1
Split Flow
40 mL/min
Gas Saver (Off)
On
20 mL/min
After:
2 min

5.2.2 Columns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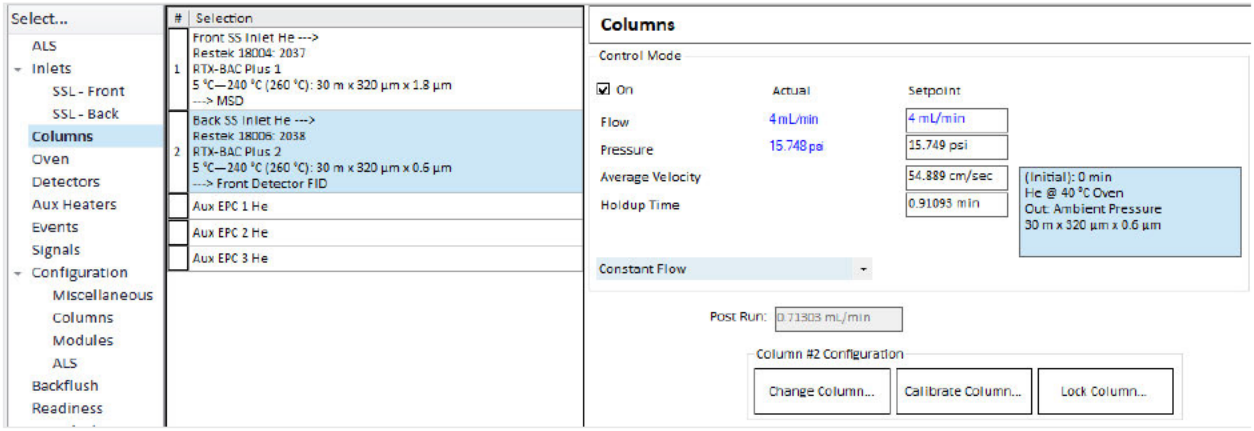
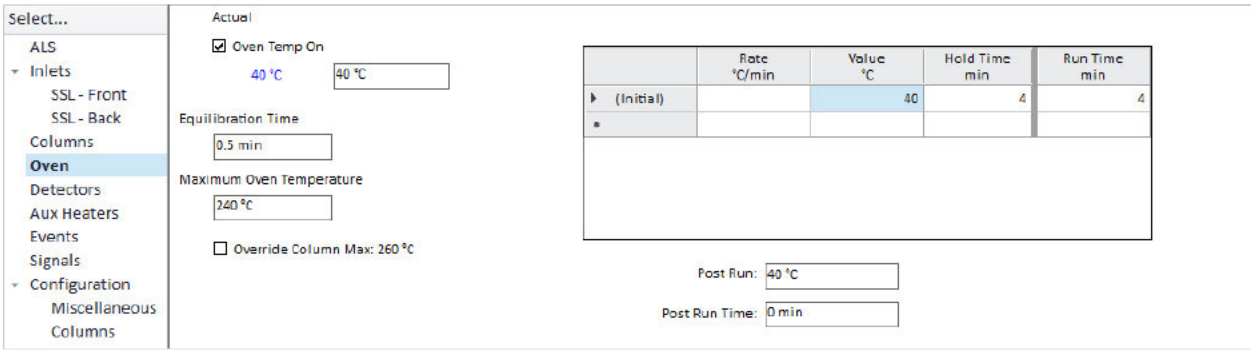
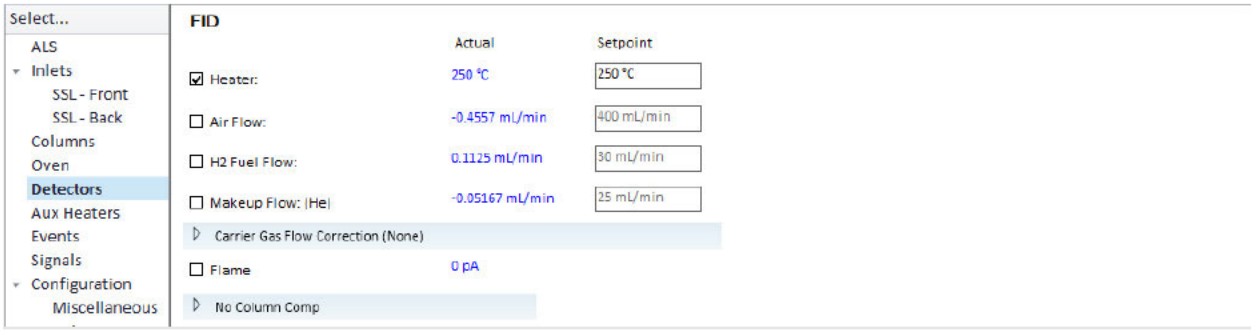
Inlets
SSL - Front
SSL - Back
Columns
Oven
Detectors
Aux Heaters
Events
Signals
Configuration
Miscellaneous
Columns
Modules
ALS
Backflush
Readiness
#
Selection
1
Front SS Inlet He --->
Restek 18004: 2037
RTX-BAC Plus 1
5 °C—240 °C (260 °C): 30 m x 320 µm x 1.8 µm
---> MSD
2
Back SS Inlet He --->
Restek 18006: 2038
RTX-BAC Plus 2
5 °C—240 °C (260 °C): 30 m x 320 µm x 0.6 µm
---> Front Detector FID
Aux EPC 1 He
Aux EPC 2 He
Aux EPC 3 He
Columns
Control Mode
On
Actual
Setpoint
Flow
1.42 mL/min
1.4203 mL/min
Pressure
1.433 psi
1.434 psi
Average Velocity
43.215 cm/sec
Holdup Time
1.157 min
(Initial): 0 min
He @ 40 °C Oven
Out: MSD
30 m x 320 µm x 1.8 µm
Constant Flow
Post Run:
0.44594 mL/min
Column #1 Configuration
Change Column...
Calibrate Column...
Lock Column...



5.2.3 Oven

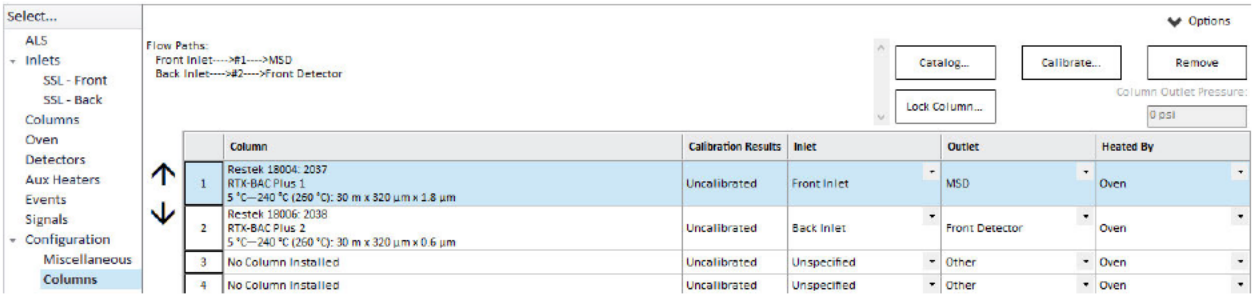


5.2.4 Detector

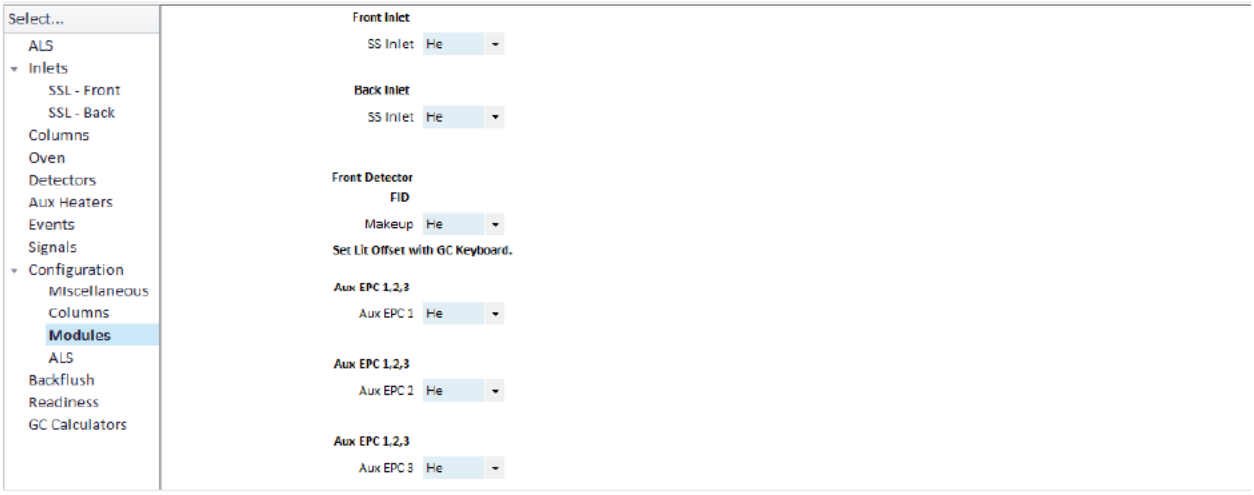


5.2.5 Aux Heaters

5.2.6 Column Configuration

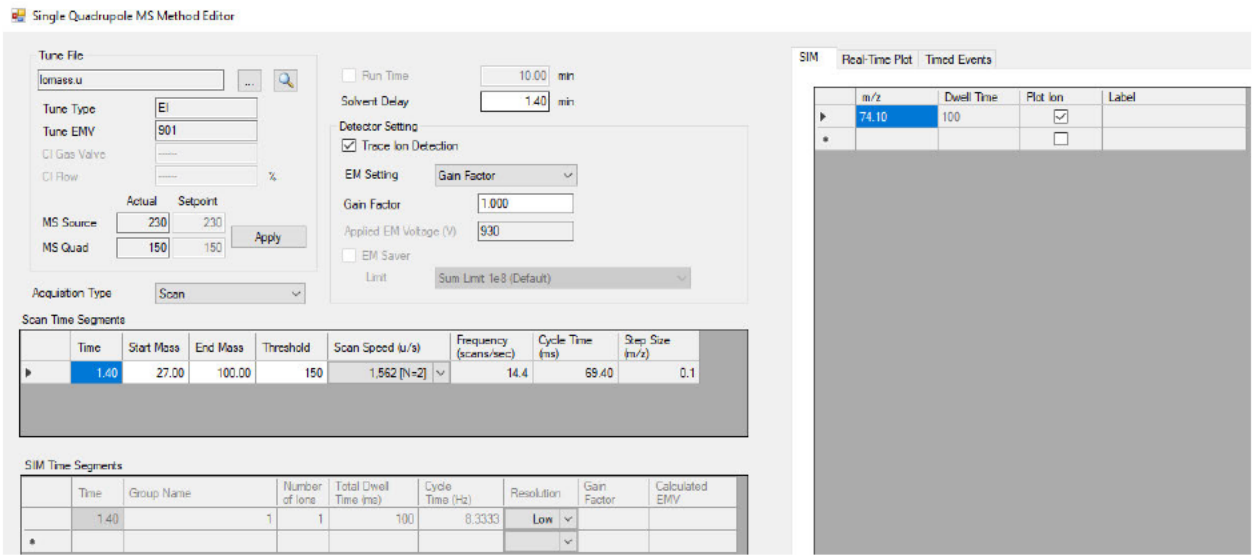


5.2.7 Module Configuration

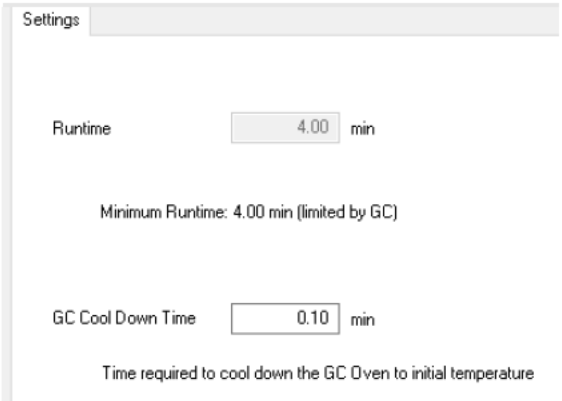


5.2.8 GC Readiness

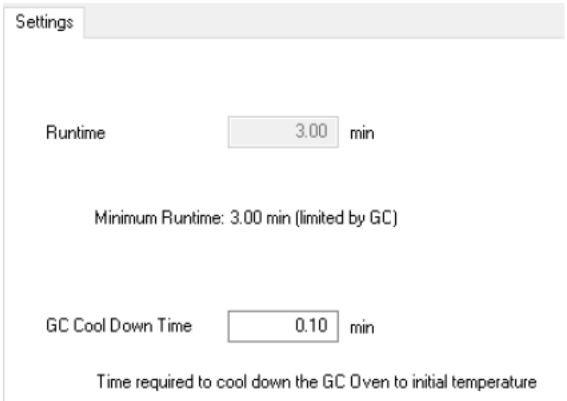
5.2.9 Quadrupole Settings



5.3 Gerstel AutoSampler Settings (FID and MSD)



MSD (Screening)



FID (Confirmation)

Headspace Injection Settings | Options

Syringe Settings

Syringe	2500ul 65mm HS
Syringe Temp. (°C)	65
Flush Time (s)	60

Sample Preparation

Sample Mode	Standard
— Heating and Incubation	
Incubator	Agitator 1
☑ Incubation Temp. (°C)	50 (50)
Incubation Time (min)	15.00
Agitator On Time (s)	10
Agitator Off Time (s)	1
Agitator Speed (rpm)	250

Sample

Inj. Volume (µL)	1000.0
Inj. Speed (µL/s)	1500.00
Pullup Delay (s)	0
Fill Volume (µL)	1000.0
Fill Strokes	3
Fill Speed (µL/s)	500.00
Pre Inj. Delay (s)	0
Post Inj. Delay (s)	0
Inj. Penetration (mm)	25.00
Sample Tray Type	R60/10-CVM
Vial Penetration (mm)	22.00

Headspace Injection Settings | Options

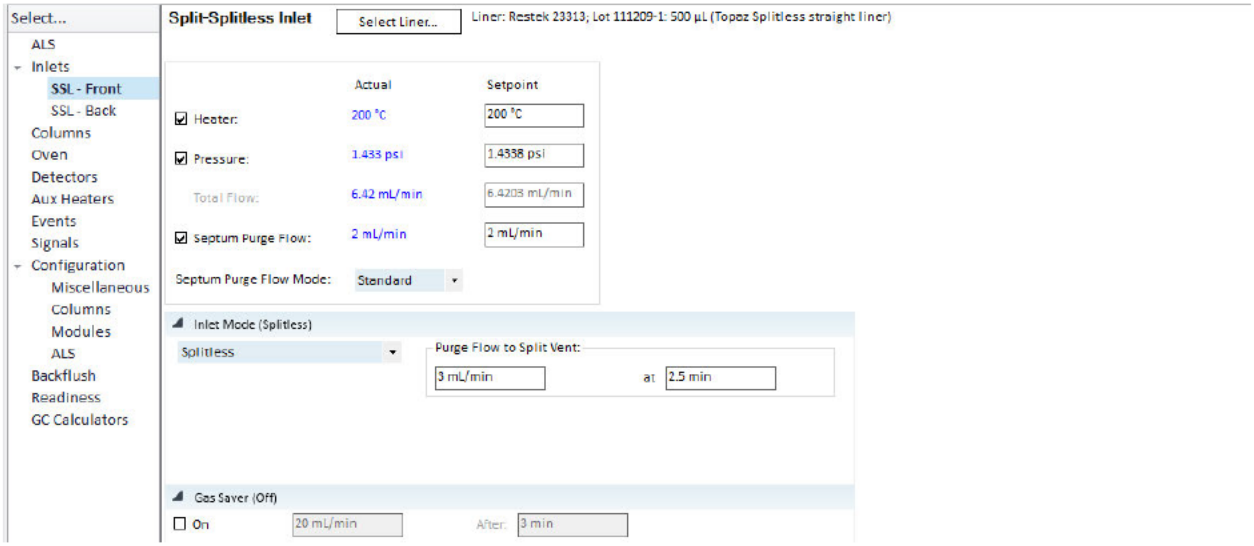
Multiple Headspace Sample Enrichment (MHSE) and/or Pressurize

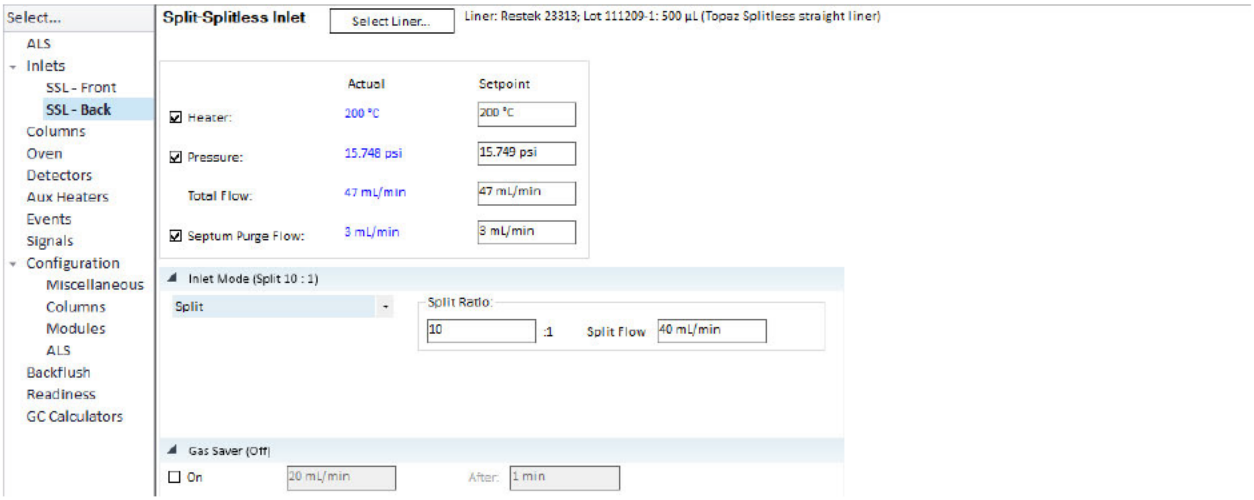
☐ Pressurize Sample

Injections per Run	1
Delay Time (min)	1.00

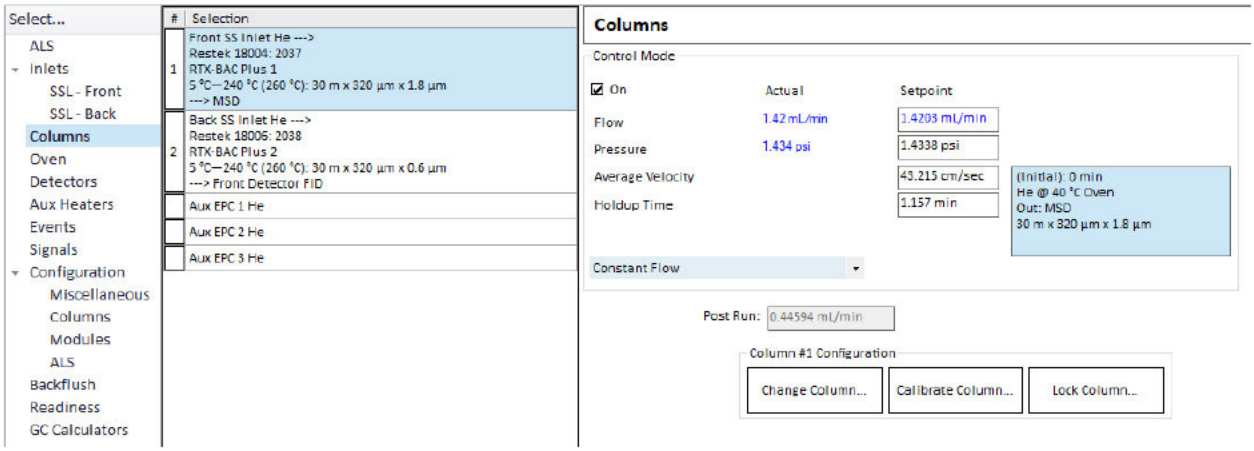
5.4 Flame Ionization Method (Confirmation)

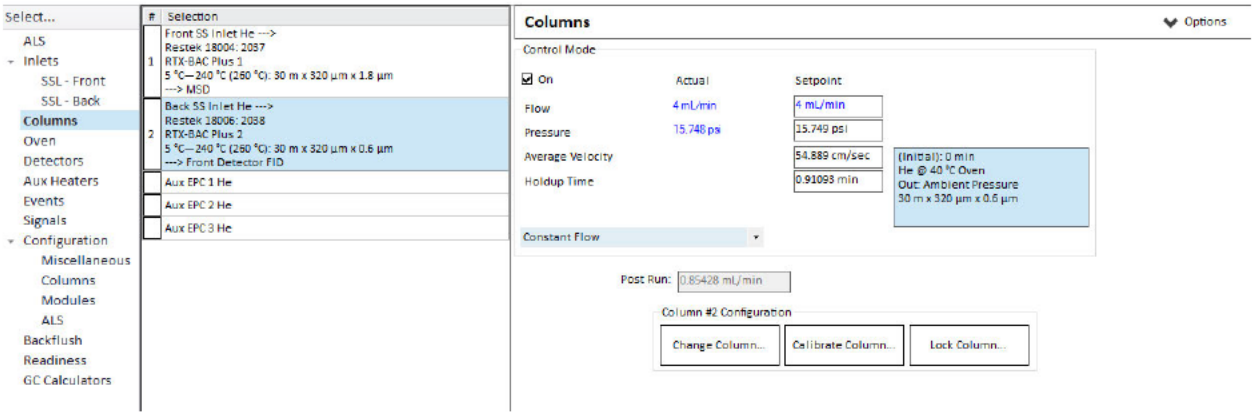
5.4.1 Inlets



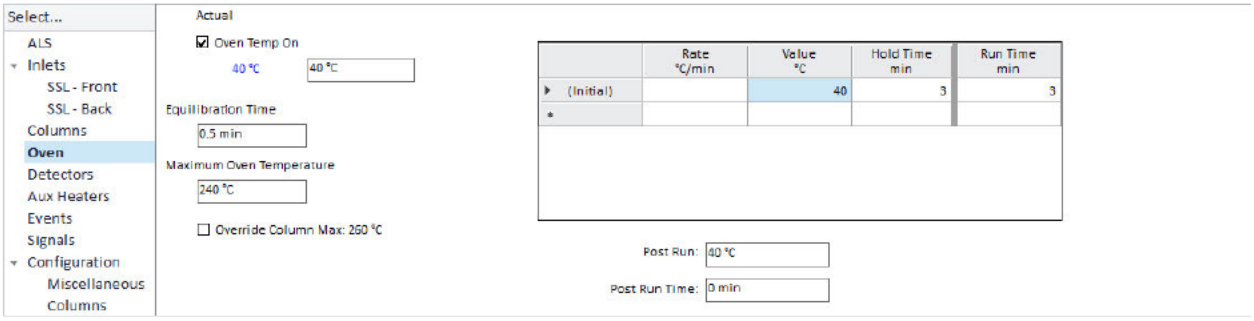


5.4.2 Columns

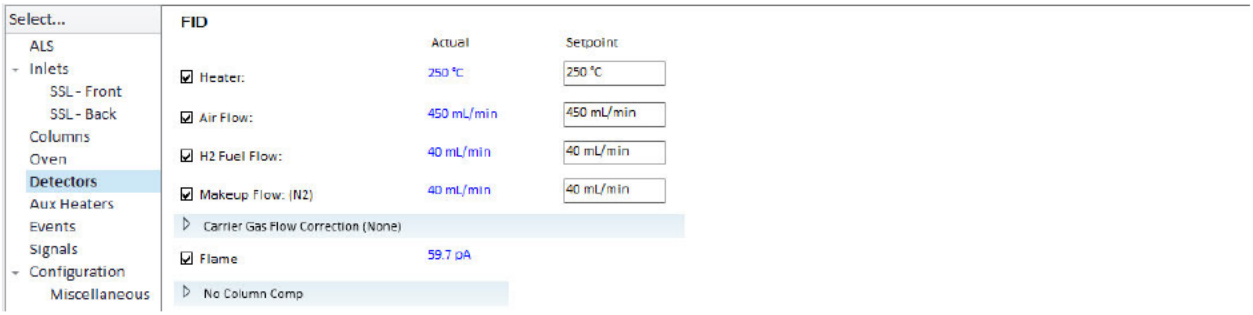




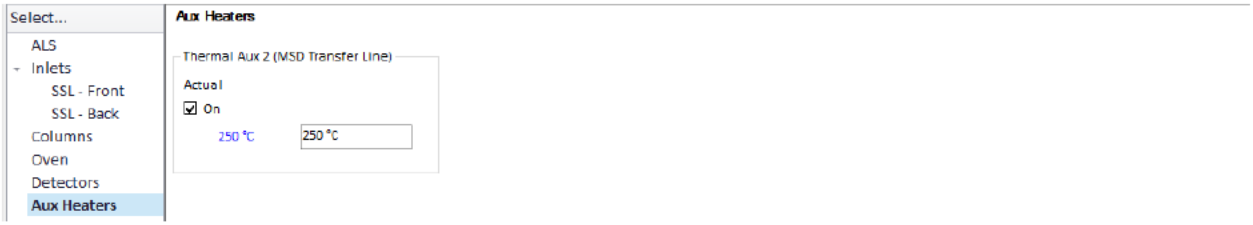
5.4.3 Oven



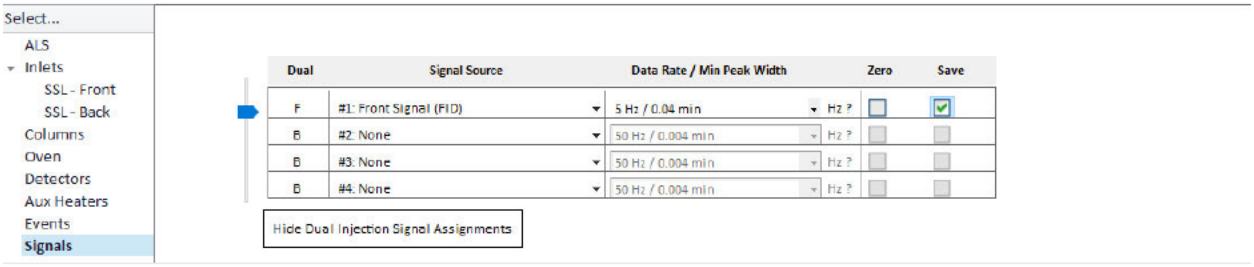
5.4.4 Detector



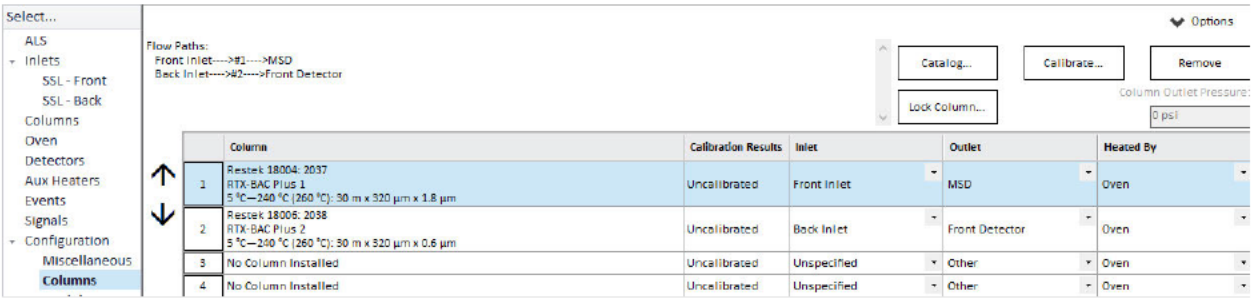
5.4.5 Aux Heater



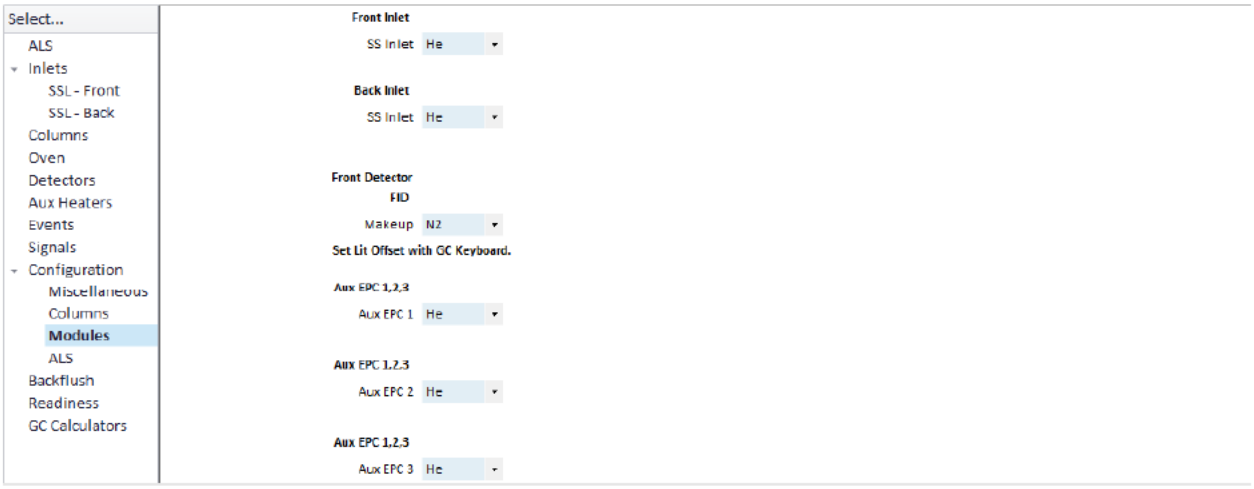
5.4.6 Signals



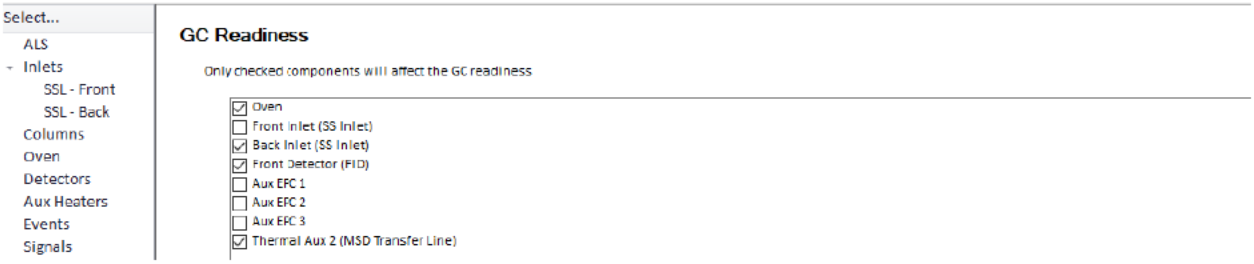
5.4.7 Column Configuration



5.4.8 Module Configuration



5.4.9 GC Readiness



5.5 Software

The below software is used to perform the analyses. Significant software revisions will be updated as appropriate.

- Agilent Masshunter Workstation
- Agilent Masshunter Workstation Quantitative Analysis
- Gerstel Maestro Version
- Gerstel MPS Firmware

6 Equipment/Materials/Reagents

6.1 Equipment and Materials

Item	Description
GC/MS with Headspace Autosampler	EI ionization, Gerstel autosampler
GC Columns	Restek RTX-BAC Plus 1: 30m X 0.32mm X 1.8 µm PN 18004 Restek RTX-BAC Plus 2: 30m X 0.32mm X 0.6 µm PN 18006
Inlet Liner*	Restek 1.0mm Topaz Straight Liner. PN 23333
Electronic Pipettor*	Eppendorf Xplorer Plus, single channel, 50-1000µL range
Pipette Tips*	Biotix™ uTIP™ Filter Pipette Tips for Universal Pipettes, Standard. PN M12509FC96
Headspace vial cap crimper	Standard, 10mL
Headspace vials*	Gerstel, crimp cap vials, 10 mL, 100 pack. PN 093640-005-00.
Headspace vial caps, magnetic*	Gerstel, crimp caps with septum for vials, 100 pack. PN 093640-063-00
Routine Laboratory Glassware and supplies	Volumetric flasks (50, 100 and 1000 mL), pipettes, disposable tissue grinder
Laboratory Balance	Standard, ≥0.1g resolution. Traceable.
*use of an equivalent product is allowable	

6.2 Chemicals

Item	Supplier*	Description	Part Number*
t-butanol	Sigma-Aldrich	~ACS/Reagent Grade	360538
Ethanol	Sigma-Aldrich	~HPLC grade	E7023
Methanol	Fisher Scientific	~HPLC grade	A454
Isopropanol	Fisher Scientific	~HPLC grade	A451
Acetone	Fisher Scientific	~HPLC grade	A949
Deionized water	Laboratory supplied	18.2 MΩ•cm	N/A
*use of an equivalent product is allowable			

6.3 Prepared Mixtures and Solvents

Depending upon the batch size, the absolute amounts may be adjusted so long as the ratios of components are maintained.

6.3.1 Stock Sample Diluent (1.0 g/100mL)

Step	Action	Amount	Component/Information
1	Acquire	1	Volumetric flask, 100 mL
2	Add	~90 mL	Deionized water
3	Add	1.0 g	t-butanol
4	QS	100 mL	Deionized water
5	Mix		
6	Transfer		Glass container
7	Storage		Refrigerated or ambient
8	Stability		≥ 6 months
9	Prepares	100 mL	(20 Sample Diluent preparations)

6.3.1 Sample Diluent (0.005 g/100mL)

Step	Action	Amount	Component/Information
1	Acquire	1	Volumetric flask, 1000 mL
2	Add	5.0 mL	Stock Sample Diluent
3	QS	1000 mL	Deionized water
4	Mix		
5	Transfer		Glass container, tightly sealed
6	Storage		Ambient. Smaller satellite container may also be used.
7	Stability		≥ 6 months
8	Prepares	1000 mL	(1250 analyses)

6.3.2 TOX200 Stock System Suitability Sample (0.100 g/100mL)

Step	Action	Amount	Component/Information
1	Acquire	1	Volumetric flask, 50 mL
2	Add	~25 mL	Deionized Water
3	Add	0.064 mL	Each of stock ethanol, acetone, isopropanol, methanol
3	QS	50 mL	Deionized water
4	Mix		
5	Transfer		Glass container, tightly sealed
6	Storage		Refrigerated.
7	Stability		≥ 12 months
8	Prepares	50 mL	Of stock material

6.3.3 TOX200 System Suitability Sample (0.010 g/100mL, S^3)

Step	Action	Amount	Component/Information
1	Acquire	1	Volumetric flask, 50 mL
2	Add	~25 mL	Deionized Water
3	Add	5 mL	Stock System Suitability Sample
3	QS	50 mL	Deionized water
4	Mix		
5	Transfer		Glass container, tightly sealed
6	Storage		Refrigerated.
7	Stability		≥ 12 months
8	Prepares	50 mL	(500 analyses)

7 Standards and Controls

7.1 Primary Standards and Controls

Analyte	Supplier*	Description	Part Number*
Multicomponent Volatiles	Cerilliant	C1-C6 levels containing ethanol, methanol, isopropanol and acetone at 0.010, 0.025, 0.050, 0.100, 0.200 and 0.400 g/100mL in water	A-127
Ethanol	Cerilliant	C7 level containing ethanol at 0.500 g/100mL in water	E-053
Multicomponent Volatiles	Cliniqa	Contain ethanol, methanol, isopropanol, and acetone in whole human blood (varying concentrations)	93221, 93222

*Use of an equivalent product is allowable. Store refrigerated. Stability determined by manufacturer.

7.2 System Suitability Sample (S^3)(0.010 g/100mL)

Analysis of an S^3 is used to verify system performance for both FID and MSD methods prior to case analysis.

8 Decision Criteria

The following criteria are applied through automated data analysis via Agilent Masshunter software.

8.1 FID Method

8.1.1 Integration Criteria

Analyte	RT	%RT	Criteria	Integrator	Peak Filter
Methanol	1.233	2	Close RT	Agile2	Area ≥ 3000 counts
Ethanol	1.487	2	Close RT	Agile2	Area ≥ 3000 counts
Acetone	1.607	2	Close RT	Agile2	Area ≥ 3000 counts
Isopropanol	1.697	2	Close RT	Agile2	Area ≥ 3000 counts
T-butanol	1.873	2	Close RT	Agile2	Area ≥ 3000 counts

8.1.2 Calibration Criteria

Analyte	Curve Fit	Origin	Weight	Accuracy (+/-)	Levels (g/100mL)
Methanol	Linear	Ignore	$1/x^2$	15%	0.010, 0.025, 0.050, 0.100, 0.200, 0.400
Ethanol	Linear	Ignore	$1/x^2$	10%	0.010, 0.025, 0.050, 0.100, 0.200, 0.400, 0.500
Acetone	Linear	Ignore	$1/x^2$	15%	0.010, 0.025, 0.050, 0.100, 0.200, 0.400
Isopropanol	Linear	Ignore	$1/x^2$	15%	0.010, 0.025, 0.050, 0.100, 0.200, 0.400

8.1.3 Control Criteria

Analyte	Accuracy (+/-)	Levels
Methanol	15%	Cliniqa 1, 2
Ethanol	10%	Cliniqa 1, 2
Acetone	15%	Cliniqa 1, 2
Isopropanol	15%	Cliniqa 1, 2

8.1.4 Reporting Criteria (g/100mL)

Analyte	Calculated Limit of Detection	Limit of Quantitation	Reporting Limit (Administratively Set)
Methanol	0.0019	0.010	0.005
Ethanol	0.0021	0.010	0.005
Acetone	0.0008	0.010	0.005
Isopropanol	0.0016	0.010	0.005

8.2 MSD Method

8.2.1 Integration Criteria

Analyte	RT	%RT	Criteria	Integrator	Quantifier Ion (m/z)	Peak Filter
Methanol	1.592	2	Close RT with Qualifiers	Agile2	31	Area ≥ 3000 counts
Ethanol	2.010	2	Close RT with Qualifiers	Agile2	31	S/N ≥ 10
Isopropanol	2.456	2	Close RT with Qualifiers	Agile2	45	S/N ≥ 10
Acetone	2.668	2	Close RT with Qualifiers	Agile2	43	S/N ≥ 10
T-butanol	2.909	2	Close RT with Qualifiers	Agile2	59	S/N ≥ 10

8.2.2 Qualifier Ion Criteria

Analyte	Qualifier Ion (m/z)	Relative Response	Criteria (+/-)
Methanol	29	63	15%
	32	72	15%
Ethanol	46	28	15%
	45	70	15%
Isopropanol	43	19	15%
Acetone	42	7	15%
	58	39	15%
T-butanol	57	9	15%
	41	17	15%

8.2.3 Calibration Criteria (Semi-Quantitative)

Analyte	Curve Fit	Origin	Weight	Accuracy (+/-)	Levels (g/100mL)
Methanol	Linear	Ignore	$1/x^2$	10%	0.010, 0.200
Ethanol	Linear	Ignore	$1/x^2$	10%	0.010, 0.200
Acetone	Linear	Ignore	$1/x^2$	10%	0.010, 0.200
Isopropanol	Linear	Ignore	$1/x^2$	10%	0.010, 0.200

8.2.4 Control Criteria

Analyte	Accuracy (+/-)	Level (g/100mL)
Methanol	10%	0.010
Ethanol	10%	0.010
Acetone	10%	0.010
Isopropanol	10%	0.010

8.3 Batch Acceptance

8.3.1 Control Criteria

Target analyte(s) shall not be detected in the Negative Control. Positive Control(s) shall have all target analytes identified. The software will automatically flag any control values that fail to meet the conditions in Section 8.2, including response, accuracy, retention time, and ion ratios.

8.3.2 Internal Standard

The internal standard shall be recovered for all samples. The software will automatically flag any samples that exceed 10% variation in response of the calculated mean of the calibrators for that batch.

8.3.3 Planned Action on QC Failure

If any criteria listed in Section 8 are not met, some or all of the following action steps may be appropriate (refer to TOX101 Quality Control for Toxicology Examinations for additional potential responses to QC failure(s)):

- Not reporting results from the batch and/or affected case samples
- Reaccession and reanalysis of the batch and/or affected case samples
- Performing instrument maintenance
- Remaking or using new reagents, calibrators, or control materials
- Notifying the TL who will ensure the root cause is determined and appropriate actions taken to address the issue(s)

9 Limitations

9.1 Limits of Detection and Reporting Limits

Analyte	FID LOD Calculated (g/100mL)	MSD Reporting Limit (g/100mL)
Ethanol	0.0021	0.010
Methanol	0.0019	0.010
Acetone	0.0008	0.010
Isopropanol	0.0016	0.010

9.2 Limit of Quantitation (FID)

Analyte	Calculated (g/100mL)	Quantitation Reporting Limit (g/100mL)
Ethanol	0.0065	0.0100
Methanol	0.0057	0.0100
Acetone	0.0025	0.0100
Isopropanol	0.0049	0.0100

9.3 Linear Range (FID)

Analyte	(g/100mL)
Ethanol	0.010 – 0.500
Methanol	0.010 – 0.400
Acetone	0.010 – 0.400
Isopropanol	0.010 – 0.400

9.4 Precision (n=52 per level)(FID, initial values)

Analyte	Low (%)	High (%)
Ethanol	1.83	1.60
Methanol	1.92	1.81
Acetone	5.83	5.13
Isopropanol	2.15	1.70

9.5 Processed Sample Stability

When secured in unanalyzed, sealed headspace vials, samples are stable for at least 15 days in refrigerated conditions. Once the septa on a vial is punctured, the analyte response will decrease, becoming less stable after 24 hours. Samples may be reanalyzed for up to 24 hours after the initial analysis for screening purposes. Quantitative analyses will not be reanalyzed.

9.6 Interferences

No interferences have been identified for this method.

9.7 Interpretation

Ethanol is normally present in the human body at low levels (<0.001 g/100mL) due to bacterial fermentation in the intestines. Ethanol can also be produced because of putrefactive processes, attributed to post-mortem processes and/or sample storage conditions. Consequently, caution should be exercised in the interpretation of low ethanol results (<0.04 g/100mL) in post-mortem cases.

10 Sampling

Representative portions of the specimens are obtained. See TOX101 for further details.

11 Calculations

11.1 MSD Screening

Calibration is linear with $1/x^2$ weighting. A two point semi-quantitative curve provides an estimated analyte concentration. For additional guidance, refer to Section 8.2.3 and TOX101.

11.2 FID Confirmation

Calibration is linear with $1/x^2$ weighting. A six or seven point calibration curve is used to provide quantitative results. Case samples are analyzed in duplicate and the values are averaged. For additional guidance, refer to Sections 8.1.2 and TOX101.

11.3 Characterization of Whole Blood Controls

For commercial volatiles controls, each newly acquired lot of control will be analyzed at least 20 times in a minimum of four batches. The initial target value for the new control will be the average of these 20 values. At least every six months, the accepted target value will be recalculated as the average value from all runs to date, excluding any failed analytical runs. Should the recalculated target value of the control ever exceed ± 5% of the nominal value for ethanol (or ± 0.005 g/100mL, whichever is greater), or ±10% of the nominal value for any of the other volatiles, the control may be degrading and a new lot should be purchased and characterized. The Technical Leader will ensure that a database of the lot performance of each lot of volatiles control is maintained.

12 Measurement Uncertainty

The critical sources of measurement uncertainty in this procedure include:

- historical random uncertainty of repeated measurements
- accuracy of the pipette used to deliver the sample
- accuracy of the pipette used to deliver the calibrators
- uncertainty in the concentration of the calibration standards
- precision of the delivery of internal standard

The measurement uncertainty will be estimated and reported following the *Chemistry Unit Procedures for Estimating Measurement Uncertainty* standard operating procedure (CUQA 13). Information used to derive uncertainty measurements will be tracked in an electronic database.

13 Reporting of Results

13.1 MSD Screening

Analytes that are identified above the estimated 0.010 g/100mL reporting limit are confirmed by FID quantitative analysis prior to reporting. If no analytes are identified, then the results are reported as not detected.

13.2 FID Confirmation

Analytes are reported according the following scheme:

Scenario	
Quantitated ≥ 0.010 g/100mL	[analyte]: [concentration] [expanded measurement uncertainty]
Quantitated ≥0.005 < 0.010 g/100mL	[analyte]: less than 0.010 g/100mL
Quantitated <0.005 g/100mL	[analyte]: not detected
Quantitated > highest calibrator	[analyte]: > [highest calibrator] g/100mL

13.3 Reporting of Quantitative Values

Replicate values are averaged. This average value is truncated to three digits. The method's expanded uncertainty value is rounded up to the third decimal place. The current k value and a coverage probability of 99.7% are also expressed.

> Example:
> Ethanol: 0.051 +/- 0.006; Acetone: 0.097 +/- 0.012; Methanol: not detected; Isopropanol: not detected; reported units g/100mL (grams per 100 milliliters). Uncertainty values reported at a coverage probability of 99.7% (k=3).

14 Safety

Take standard precautions for the handling of chemicals and biological materials. Refer to the *FBI Laboratory Safety Manual* for guidance.

15 References

Dubowski, K.M., *Manual for Analysis of Ethanol in Biological Liquids*, 1977.

Garriott, James, *Medicolegal Aspects of Alcohol*, 6th ed., Lawyers and Judges Publishing: Tucson, AZ, 2015.

Rev.#	Issue Date	History
15	09/15/2020	References - Removed internal document references 5k - Updated preparation and stability 6g - Replaced 'Control Tracking Supervisor' with 'Technical Leader or designee". Clarified language regarding setting target values and recalculations of target values. 3, 8a, 8d - Screening by GC/MS, quant/confirm by GC/FID 11 - Added that replicate values are averaged. Instr. App. - Corrected typographical error on instrument appendix.
16	12/15/2020	Complete document reformat.

Approval Redacted - Signatures on File

Toxicology
Technical Leader: Date: 12/14/2020

Chemistry Unit Chief: Date: 12/14/2020